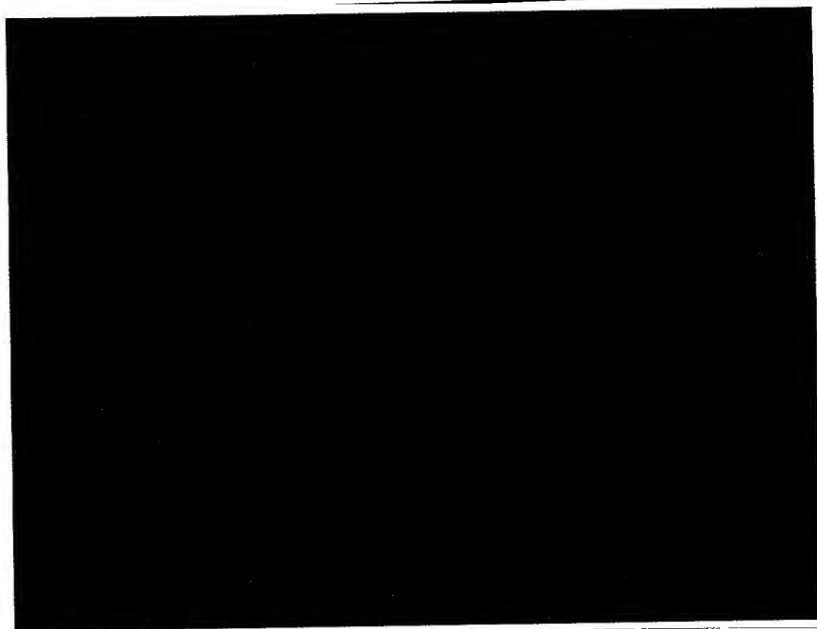


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**GFP-LCA (wt)**

Figure 1

**GFP-TLCA**

Figure 2

|     |                                     |                                       |                                      |  |                                      |
|-----|-------------------------------------|---------------------------------------|--------------------------------------|--|--------------------------------------|
| 1   | PFVVKQFN <sup>Y</sup> K             | DPVNGVDIAY                            | IKIPNVGQMQ                           | PVKAFKIH <sup>N</sup> K                            | IWVIPERDTF                           |
| 51  | TNPEEGDLNP                          | PPEAKQVPVS                            | Y <sup>Y</sup> DSTYLSTD              | NEKDN <sup>Y</sup> LKGV                            | TKL <sup>F</sup> ERIYST              |
| 101 | DLGRMLLTSI                          | VRGIPFWGGS                            | TIDTELKVID                           | TNCIN <sup>V</sup> IQPD                            | GSYRSEELNL                           |
| 151 | VIIGPSADII                          | QFECKSFGHE                            | VLNLTRNG <sup>Y</sup> G              | STQYIRFSPD   | FTFGFEESLE                           |
| 201 | VDTNPLLGAG                          | KFATDPAVTL                            | AHELIHAGHR                           | LYGIAINPNR   | VFKVNTNAYY                           |
| 251 | EMSGLEVSFE                          | ELRTFGGHDA                            | KFIDSLQENE                           | FRLY <sup>Y</sup> Y <sup>N</sup> KN <sup>F</sup> K | DIAS <sup>T</sup> LNKAK              |
| 301 | SIVGTTASLQ                          | Y <sup>M</sup> MKNV <sup>F</sup> KEKY | LLSEDTSGKF                           | SVDKLK <sup>F</sup> DKL                            | Y <sup>K</sup> ML <sup>T</sup> EIYTE |
| 351 | DNFVKFFKVL                          | NRKT <sup>Y</sup> LN <sup>F</sup> DK  | AVFKINI <sup>V</sup> PK              | VNYTI <sup>Y</sup> DC <sup>F</sup> N               | LRNTNLAANF                           |
| 401 | NGQNT <sup>E</sup> INN <sup>M</sup> | NFTKLKNFTG                            | L <sup>F</sup> EFYKLL <sup>C</sup> V | RGII <sup>T</sup> SK                               |                                      |

Figure 3

## GFP-LCA (AA mut)

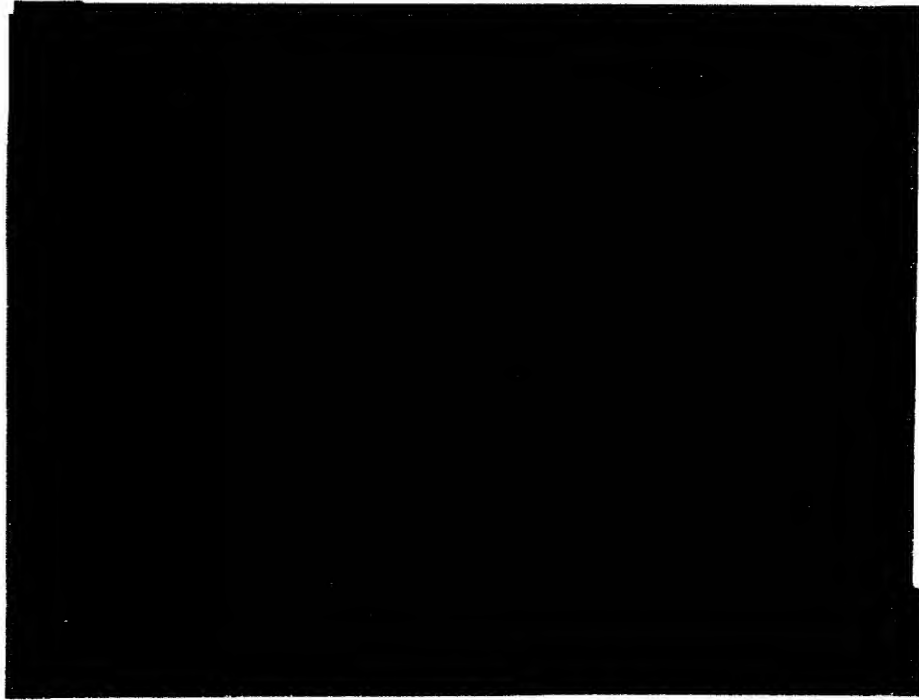


Figure 4

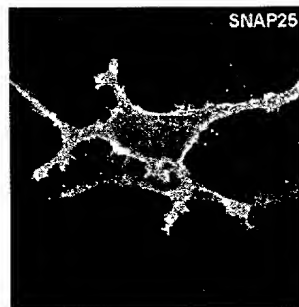
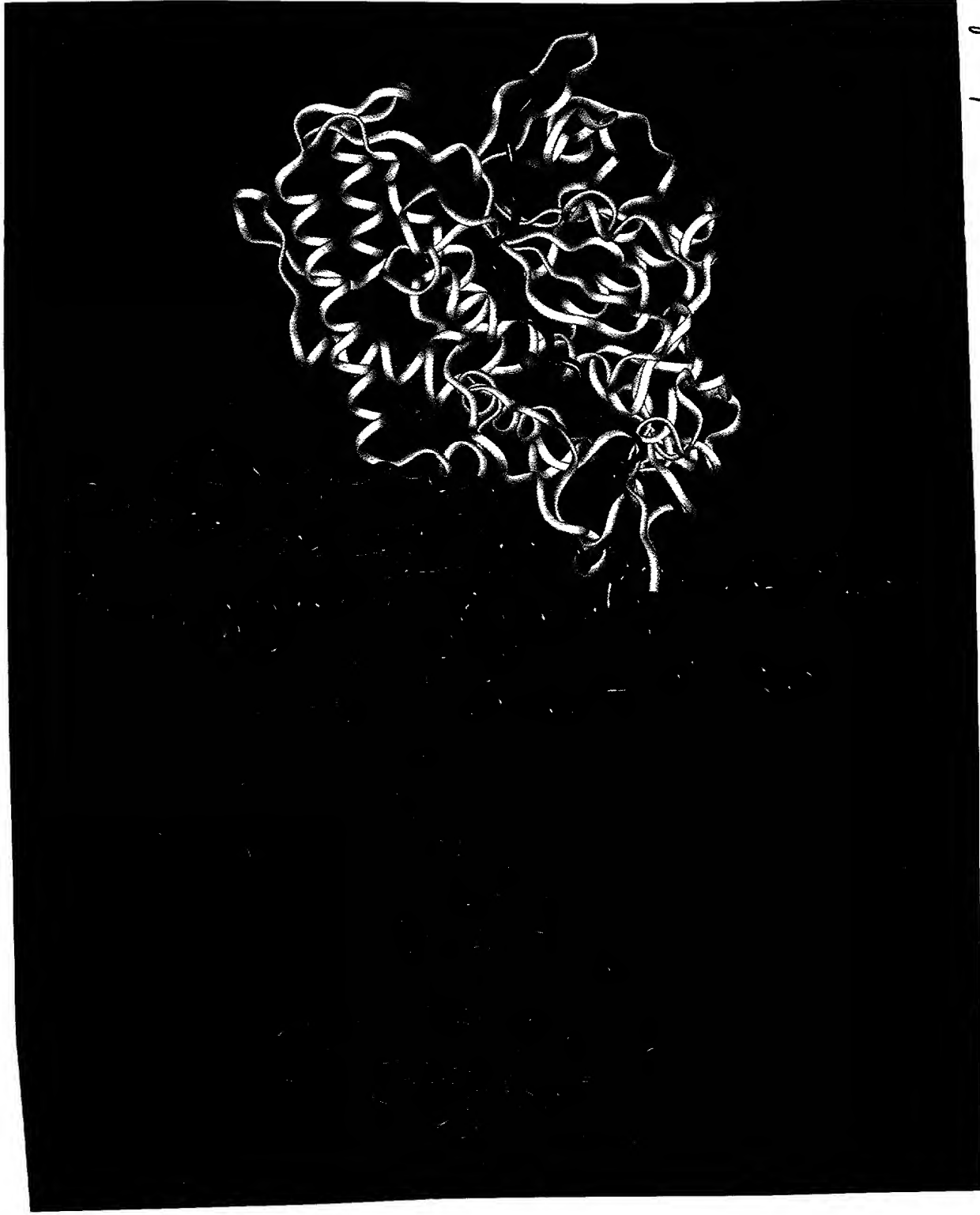


Figure 5

161220: 0451550

# BOTULINUM TOXIN

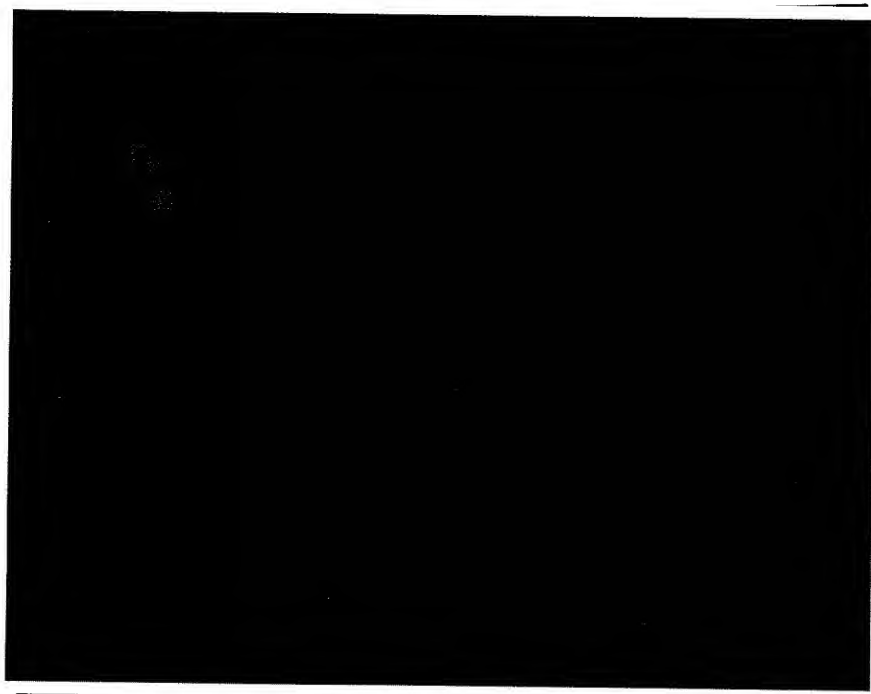
## TYPE A X-RAY STRUCTURE



Green = binding domain  
Red = translocation domain  
Yellow = protease domain

Figure 6

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**GFP-LCB(wt)**

Figure 7

|                    |       |   |        |        |       |        |      |    |     |      |      |    |      |    |    |    |    |    |    |      |    |     |     |
|--------------------|-------|---|--------|--------|-------|--------|------|----|-----|------|------|----|------|----|----|----|----|----|----|------|----|-----|-----|
| BoNT-A_Halla_LC    | 1     | - | EFVNKQ | ENYKDP | ANGVD | JAYIKI | ENVG | QM | QPV | KAFK | HNKI | QV | PERD | IT | NP | PE | GD | LN | PP | PEAK | QV | PVS | YYL |
|                    | (1)   |   |        |        |       |        |      |    |     |      |      |    |      |    |    |    |    |    |    |      |    |     |     |
|                    | (1)   |   |        |        |       |        |      |    |     |      |      |    |      |    |    |    |    |    |    |      |    |     |     |
|                    | (1)   |   |        |        |       |        |      |    |     |      |      |    |      |    |    |    |    |    |    |      |    |     |     |
| BoNT-B_Danish_I_LC | 1     |   |        |        |       |        |      |    |     |      |      |    |      |    |    |    |    |    |    |      |    |     |     |
|                    | (1)   |   |        |        |       |        |      |    |     |      |      |    |      |    |    |    |    |    |    |      |    |     |     |
|                    | (1)   |   |        |        |       |        |      |    |     |      |      |    |      |    |    |    |    |    |    |      |    |     |     |
|                    | (1)   |   |        |        |       |        |      |    |     |      |      |    |      |    |    |    |    |    |    |      |    |     |     |
| BoNT-A_Halla_LC    | 75    |   |        |        |       |        |      |    |     |      |      |    |      |    |    |    |    |    |    |      |    |     |     |
|                    | (74)  |   |        |        |       |        |      |    |     |      |      |    |      |    |    |    |    |    |    |      |    |     |     |
|                    | (76)  |   |        |        |       |        |      |    |     |      |      |    |      |    |    |    |    |    |    |      |    |     |     |
|                    | (76)  |   |        |        |       |        |      |    |     |      |      |    |      |    |    |    |    |    |    |      |    |     |     |
| BoNT-B_Danish_I_LC | 75    |   |        |        |       |        |      |    |     |      |      |    |      |    |    |    |    |    |    |      |    |     |     |
|                    | (74)  |   |        |        |       |        |      |    |     |      |      |    |      |    |    |    |    |    |    |      |    |     |     |
|                    | (76)  |   |        |        |       |        |      |    |     |      |      |    |      |    |    |    |    |    |    |      |    |     |     |
|                    | (76)  |   |        |        |       |        |      |    |     |      |      |    |      |    |    |    |    |    |    |      |    |     |     |
| BoNT-A_Halla_LC    | 150   |   |        |        |       |        |      |    |     |      |      |    |      |    |    |    |    |    |    |      |    |     |     |
|                    | (74)  |   |        |        |       |        |      |    |     |      |      |    |      |    |    |    |    |    |    |      |    |     |     |
|                    | (76)  |   |        |        |       |        |      |    |     |      |      |    |      |    |    |    |    |    |    |      |    |     |     |
|                    | (76)  |   |        |        |       |        |      |    |     |      |      |    |      |    |    |    |    |    |    |      |    |     |     |
| BoNT-B_Danish_I_LC | 150   |   |        |        |       |        |      |    |     |      |      |    |      |    |    |    |    |    |    |      |    |     |     |
|                    | (74)  |   |        |        |       |        |      |    |     |      |      |    |      |    |    |    |    |    |    |      |    |     |     |
|                    | (76)  |   |        |        |       |        |      |    |     |      |      |    |      |    |    |    |    |    |    |      |    |     |     |
|                    | (76)  |   |        |        |       |        |      |    |     |      |      |    |      |    |    |    |    |    |    |      |    |     |     |
| BoNT-A_Halla_LC    | 225   |   |        |        |       |        |      |    |     |      |      |    |      |    |    |    |    |    |    |      |    |     |     |
|                    | (148) |   |        |        |       |        |      |    |     |      |      |    |      |    |    |    |    |    |    |      |    |     |     |
|                    | (151) |   |        |        |       |        |      |    |     |      |      |    |      |    |    |    |    |    |    |      |    |     |     |
|                    | (151) |   |        |        |       |        |      |    |     |      |      |    |      |    |    |    |    |    |    |      |    |     |     |
| BoNT-B_Danish_I_LC | 225   |   |        |        |       |        |      |    |     |      |      |    |      |    |    |    |    |    |    |      |    |     |     |
|                    | (148) |   |        |        |       |        |      |    |     |      |      |    |      |    |    |    |    |    |    |      |    |     |     |
|                    | (151) |   |        |        |       |        |      |    |     |      |      |    |      |    |    |    |    |    |    |      |    |     |     |
|                    | (151) |   |        |        |       |        |      |    |     |      |      |    |      |    |    |    |    |    |    |      |    |     |     |
| BoNT-A_Halla_LC    | 300   |   |        |        |       |        |      |    |     |      |      |    |      |    |    |    |    |    |    |      |    |     |     |
|                    | (218) |   |        |        |       |        |      |    |     |      |      |    |      |    |    |    |    |    |    |      |    |     |     |
|                    | (226) |   |        |        |       |        |      |    |     |      |      |    |      |    |    |    |    |    |    |      |    |     |     |
|                    | (226) |   |        |        |       |        |      |    |     |      |      |    |      |    |    |    |    |    |    |      |    |     |     |
| BoNT-B_Danish_I_LC | 300   |   |        |        |       |        |      |    |     |      |      |    |      |    |    |    |    |    |    |      |    |     |     |
|                    | (218) |   |        |        |       |        |      |    |     |      |      |    |      |    |    |    |    |    |    |      |    |     |     |
|                    | (226) |   |        |        |       |        |      |    |     |      |      |    |      |    |    |    |    |    |    |      |    |     |     |
|                    | (226) |   |        |        |       |        |      |    |     |      |      |    |      |    |    |    |    |    |    |      |    |     |     |
| BoNT-A_Halla_LC    | 375   |   |        |        |       |        |      |    |     |      |      |    |      |    |    |    |    |    |    |      |    |     |     |
|                    | (293) |   |        |        |       |        |      |    |     |      |      |    |      |    |    |    |    |    |    |      |    |     |     |
|                    | (300) |   |        |        |       |        |      |    |     |      |      |    |      |    |    |    |    |    |    |      |    |     |     |
|                    | (301) |   |        |        |       |        |      |    |     |      |      |    |      |    |    |    |    |    |    |      |    |     |     |
| BoNT-B_Danish_I_LC | 375   |   |        |        |       |        |      |    |     |      |      |    |      |    |    |    |    |    |    |      |    |     |     |
|                    | (293) |   |        |        |       |        |      |    |     |      |      |    |      |    |    |    |    |    |    |      |    |     |     |
|                    | (300) |   |        |        |       |        |      |    |     |      |      |    |      |    |    |    |    |    |    |      |    |     |     |
|                    | (301) |   |        |        |       |        |      |    |     |      |      |    |      |    |    |    |    |    |    |      |    |     |     |
| BoNT-A_Halla_LC    | 447   |   |        |        |       |        |      |    |     |      |      |    |      |    |    |    |    |    |    |      |    |     |     |
|                    | (367) |   |        |        |       |        |      |    |     |      |      |    |      |    |    |    |    |    |    |      |    |     |     |
|                    | (375) |   |        |        |       |        |      |    |     |      |      |    |      |    |    |    |    |    |    |      |    |     |     |
|                    | (376) |   |        |        |       |        |      |    |     |      |      |    |      |    |    |    |    |    |    |      |    |     |     |
| BoNT-B_Danish_I_LC | 447   |   |        |        |       |        |      |    |     |      |      |    |      |    |    |    |    |    |    |      |    |     |     |
|                    | (367) |   |        |        |       |        |      |    |     |      |      |    |      |    |    |    |    |    |    |      |    |     |     |
|                    | (375) |   |        |        |       |        |      |    |     |      |      |    |      |    |    |    |    |    |    |      |    |     |     |
|                    | (376) |   |        |        |       |        |      |    |     |      |      |    |      |    |    |    |    |    |    |      |    |     |     |

Figure 8

# BirA-SNAP25 Assay of rLC/A Activity

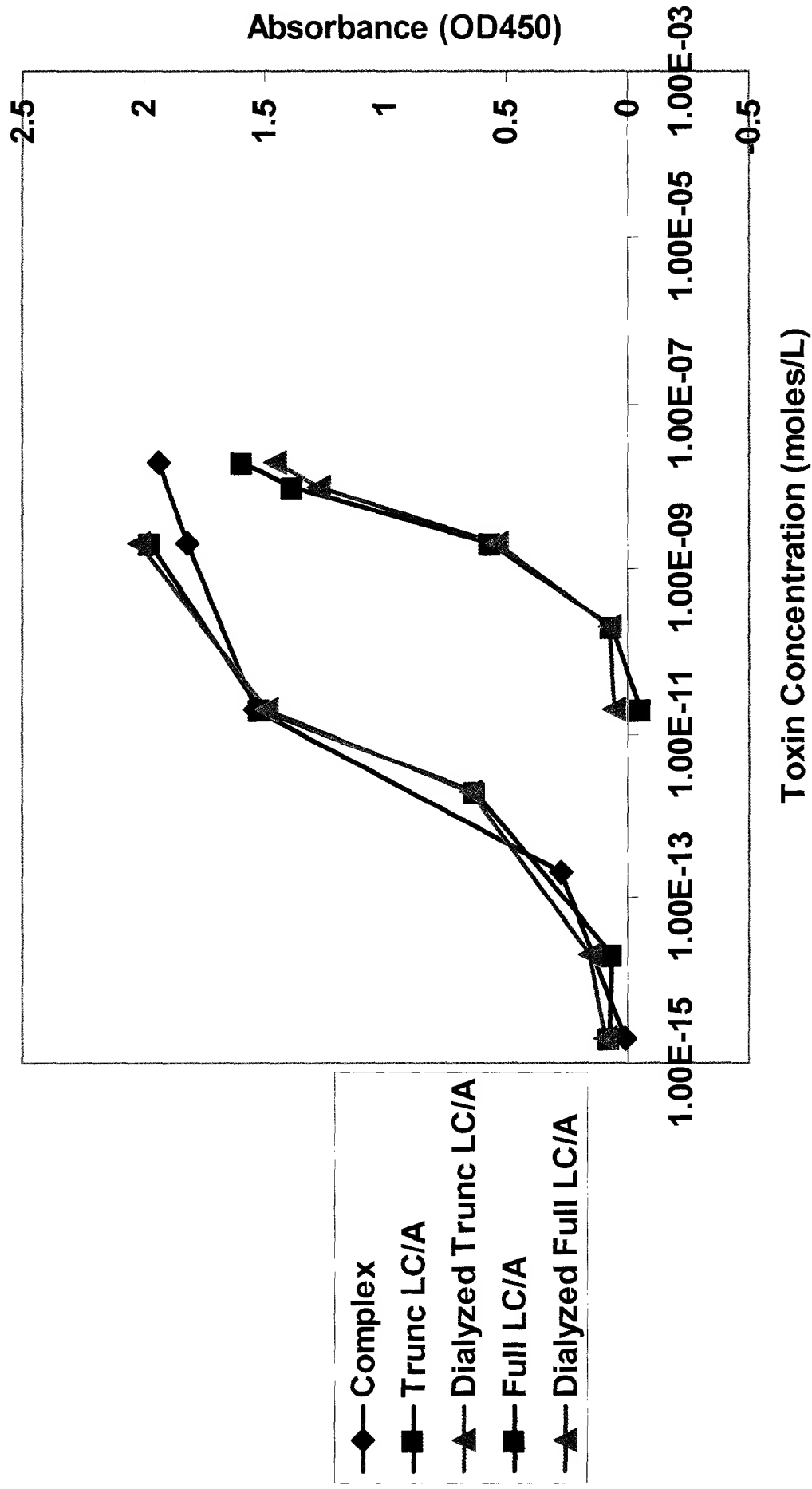


Figure 9

# Comparison of LC/A constructs expressed from E. coli for in vitro analysis

|               | <u>6xHis</u>          | <u>S-tag</u>             |                   |
|---------------|-----------------------|--------------------------|-------------------|
| Wildtype LC/A |                       |                          | PFV NKQFNYKDPV... |
| trun LC/A     | MHHHHHSSGLVPRGSGMKETA | AAKFERQHMDSPDLGTDDDDKAM  | .....YKDPV...     |
| N-His LC/A    | MHHHHHSSGLVPRGSGMKETA | AAKFERQHMDSPDLGTDDDDKAMG | SFV NKQFNYKDPV... |
| Wildtype LC/A | ...NFTKLKNFTGL        | <b>EFYKLL</b> CVRGIITSK  |                   |
| trun LC/A     | ...NFTKL.....         | TRAHHHHH                 |                   |
| N-His LC/A    | ...NFTKLKNFTGL        | <b>EFYKLL</b> CVRGIITSK  |                   |
|               |                       | <u>6xHis</u>             |                   |

- Truncated LC construct published
  - Kadkhodayan, S. et al. Prot. Exp. Purif. 2000, 19, 125-130
  - Crystal structure reported at IBRCC in Oct. 2000

Figure 10